Docket/App No.: 2313.2001-001
Title: Methods Of And Apparatus For.....
Inventors: Chris L. Jones, et al.

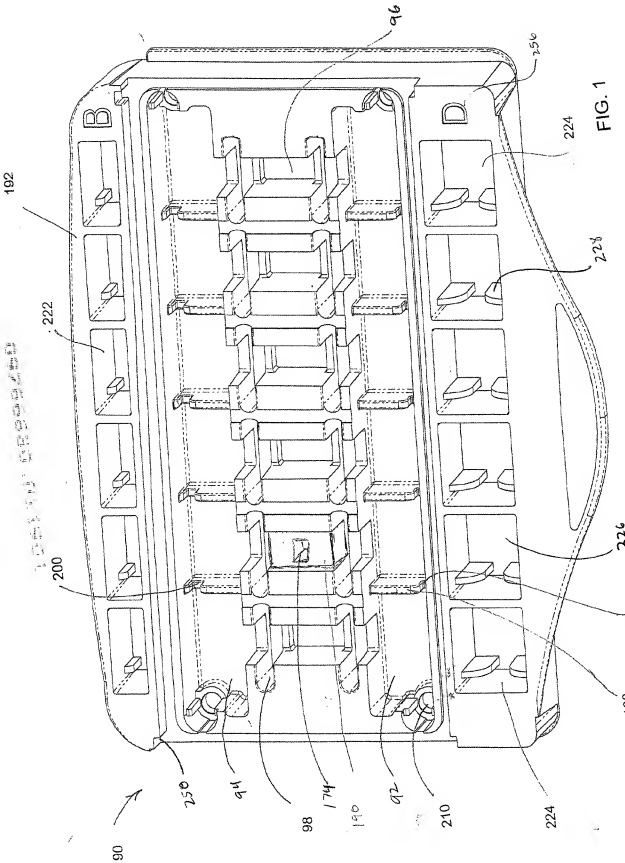


FIG. 1

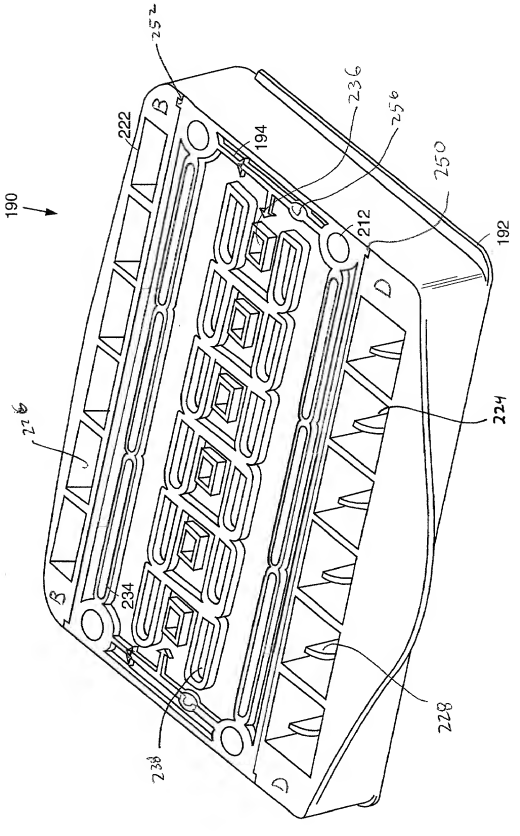


FIG. 2

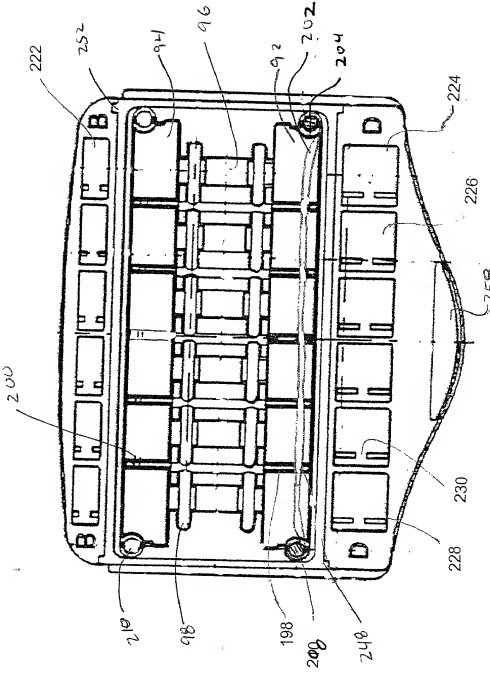


FIG. 3

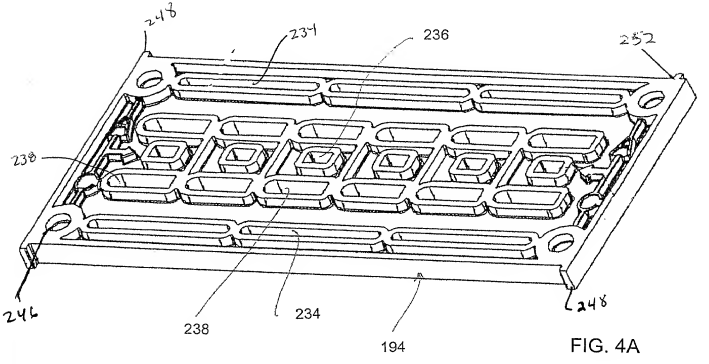
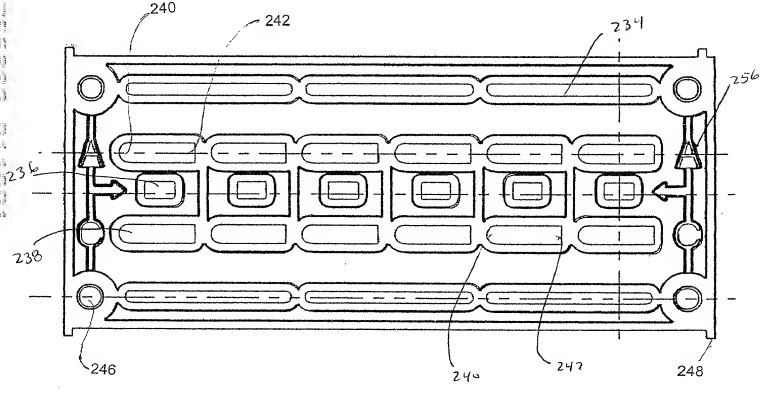

248
234
236
252
238
246
238
234
194

FIG. 4A


240
242
234
256
236
238
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240
247
248

FIG. 4B

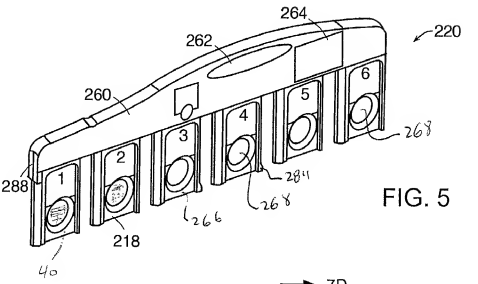


FIG. 5

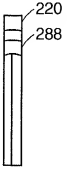


FIG. 6B

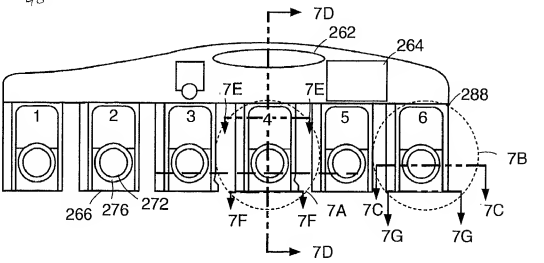


FIG. 6A

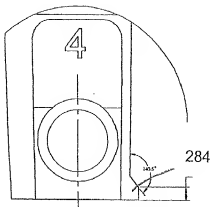


FIG. 7A

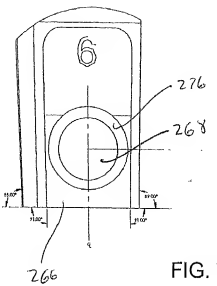


FIG. 7B

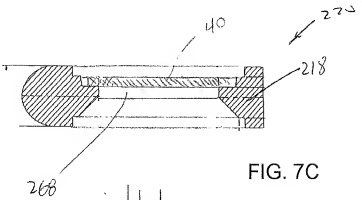


FIG. 7C

FIG. 7E

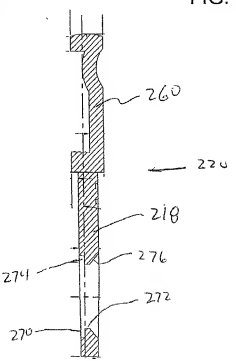


FIG. 7D

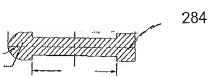


FIG. 7F

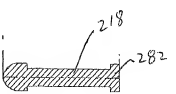


FIG. 7G

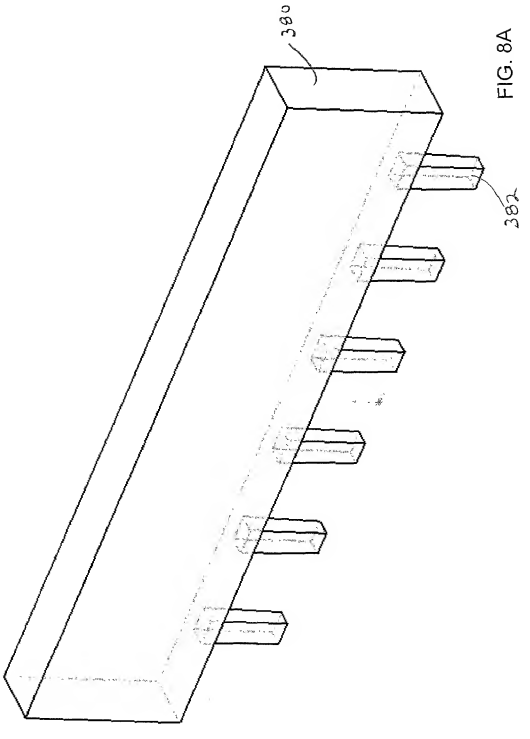


FIG. 8A

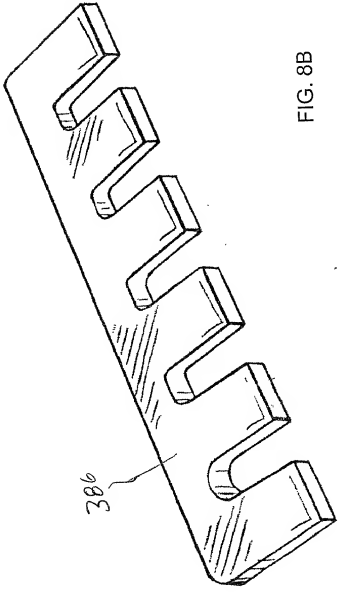


FIG. 8B

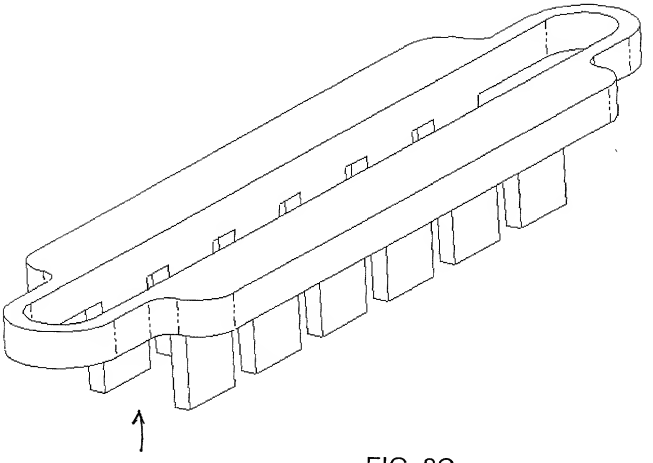


FIG. 8C

Platelets, Bacteria
130
Lysis
+ Heat,
Sample Processing
132
+ Reporter Probe
134
Hybridization
136
Capture Probe Membraine
(+)
(-)
138
Electrophoresis
Capture/Wash
140
Light
Detect
142

FIG. 9A

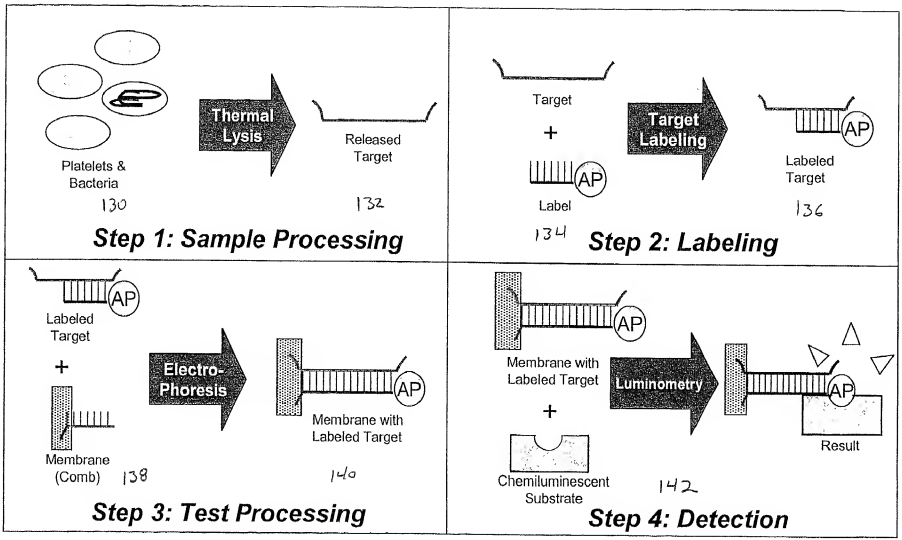
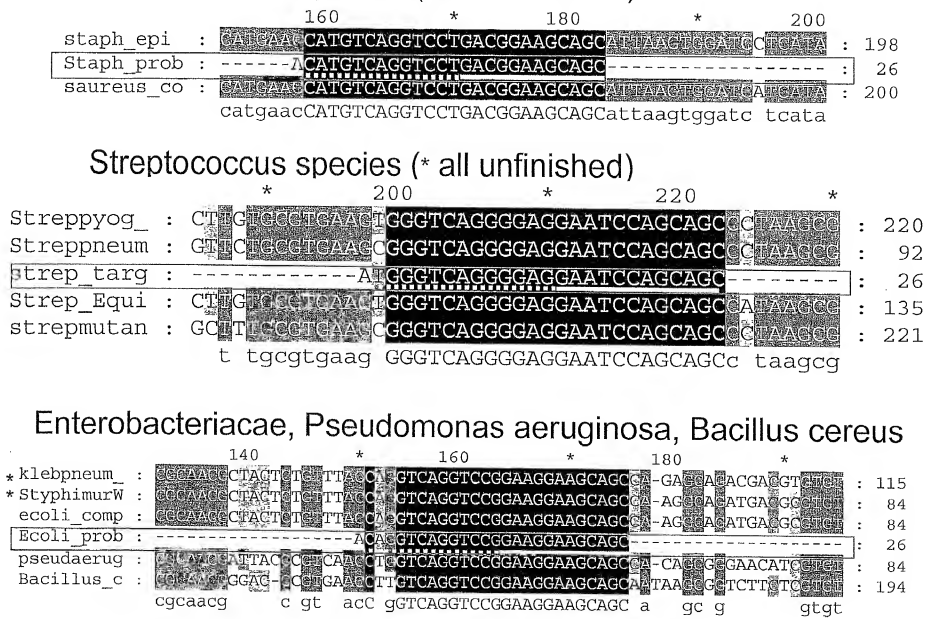


FIG. 9B



*unfinished sequence from genome centers

FIG. 9C

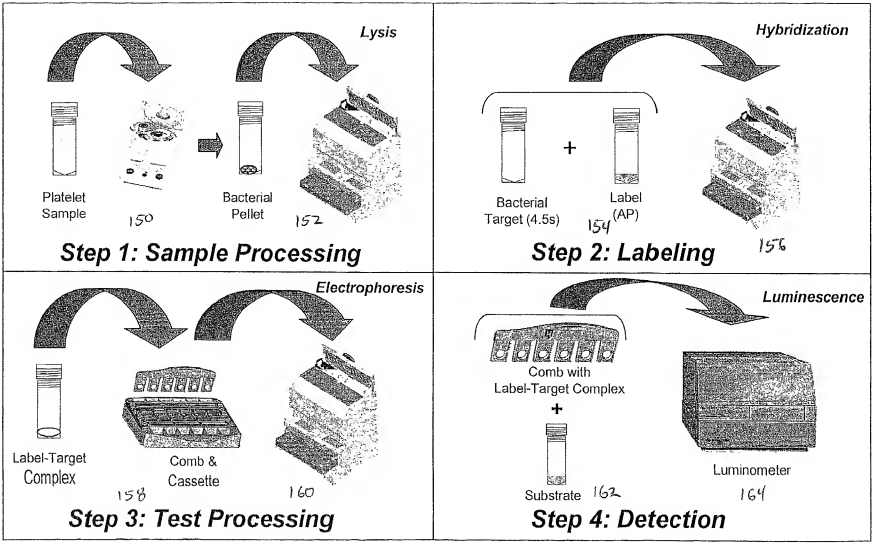

Lysis
Platelet Sample
150
Bacterial Pellet
152
Step 1: Sample Processing
Hybridization
Bacterial Target (4.5s)
Label (AP)
154
156
Step 2: Labeling
Electrophoresis
Label-Target Complex
Comb & Cassette
158
160
Step 3: Test Processing
Luminescence
Comb with Label-Target Complex
Substrate
162
Luminometer
164
Step 4: Detection

FIG. 10

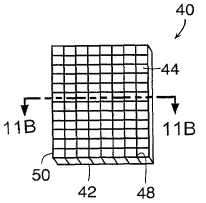


FIG. 11A

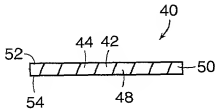


FIG. 11B

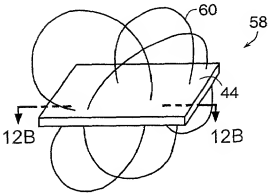


FIG. 12A

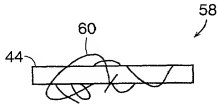


FIG. 12B

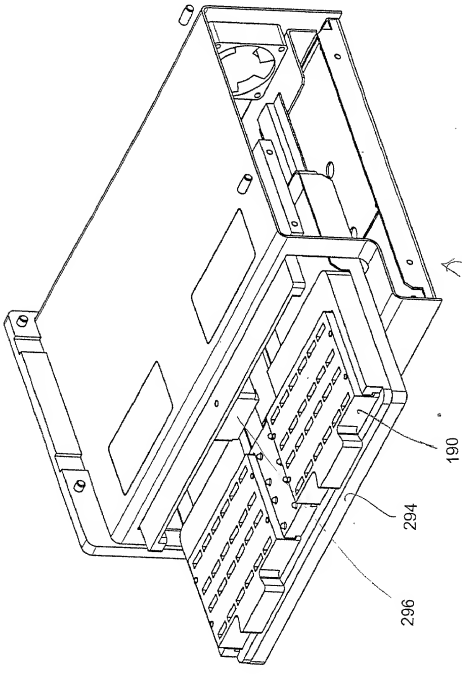


FIG. 13

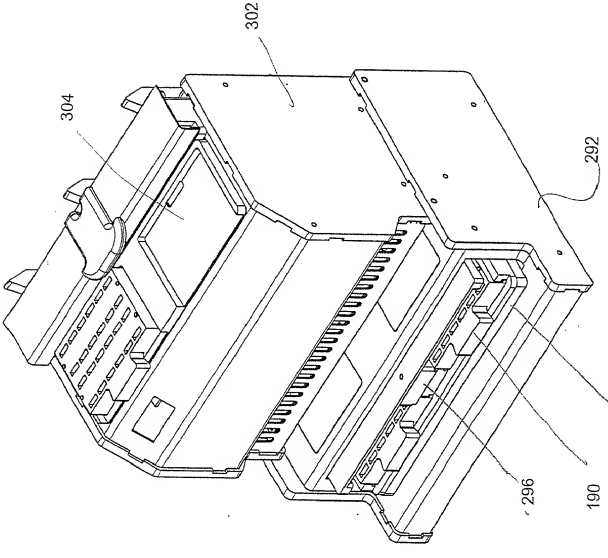


FIG. 14

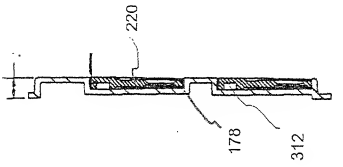


FIG. 15B

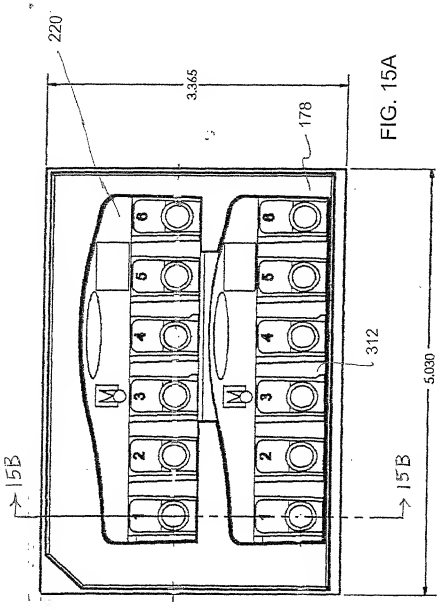


FIG. 15A

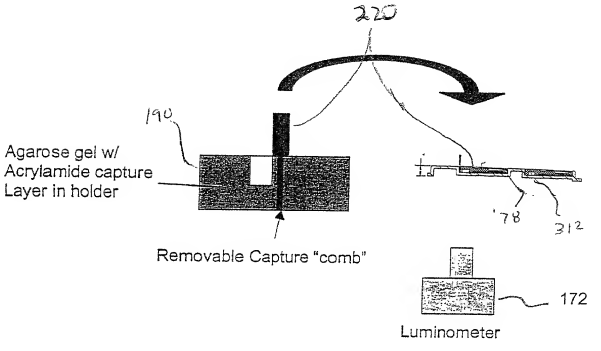
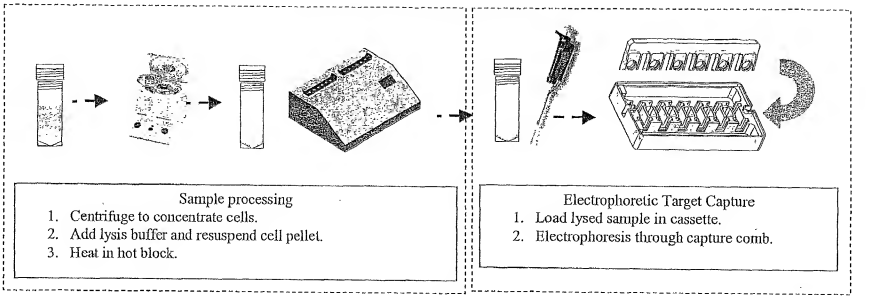

220
190
Agarose gel w/
Acrylamide capture
Layer in holder
178
312
Removable Capture "comb"
172
Luminometer

FIG. 16


Sample processing
1. Centrifuge to concentrate cells.
2. Add lysis buffer and resuspend cell pellet.
3. Heat in hot block.
Electrophoretic Target Capture
1. Load lysed sample in cassette.
2. Electrophoresis through capture comb.

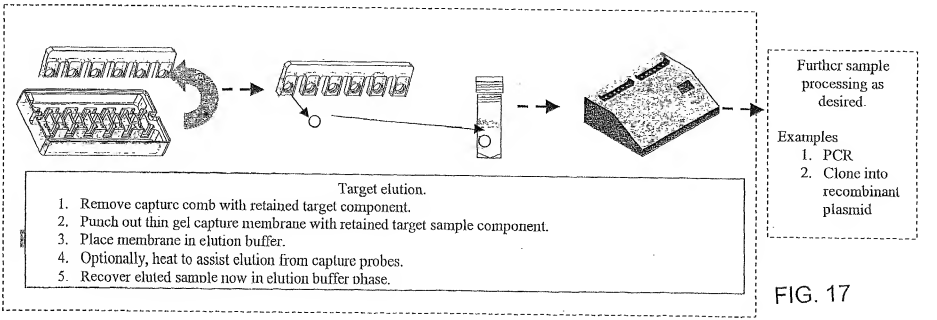

Target elution.
1. Remove capture comb with retained target component.
2. Punch out thin gel capture membrane with retained target sample component.
3. Place membrane in elution buffer.
4. Optionally, heat to assist elution from capture probes.
5. Recover eluted sample now in elution buffer phase.
Further sample processing as desired.
Examples
1. PCR
2. Clone into recombinant plasmid

FIG. 17

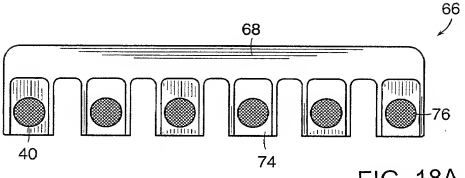


FIG. 18A

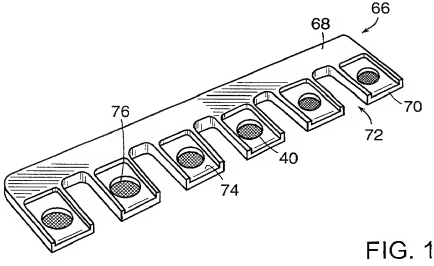


FIG. 18B

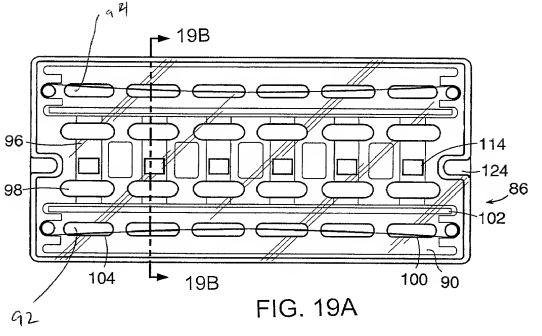


FIG. 19A

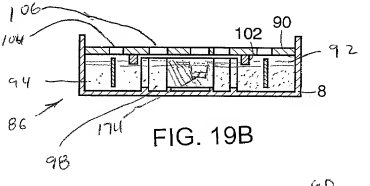


FIG. 19B

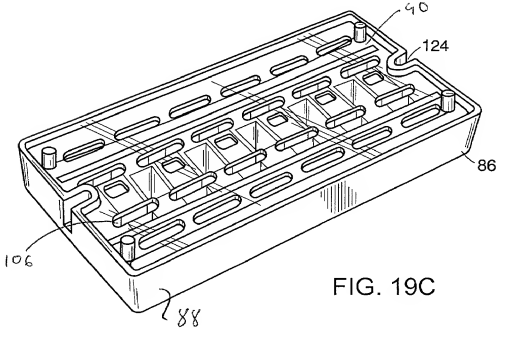


FIG. 19C

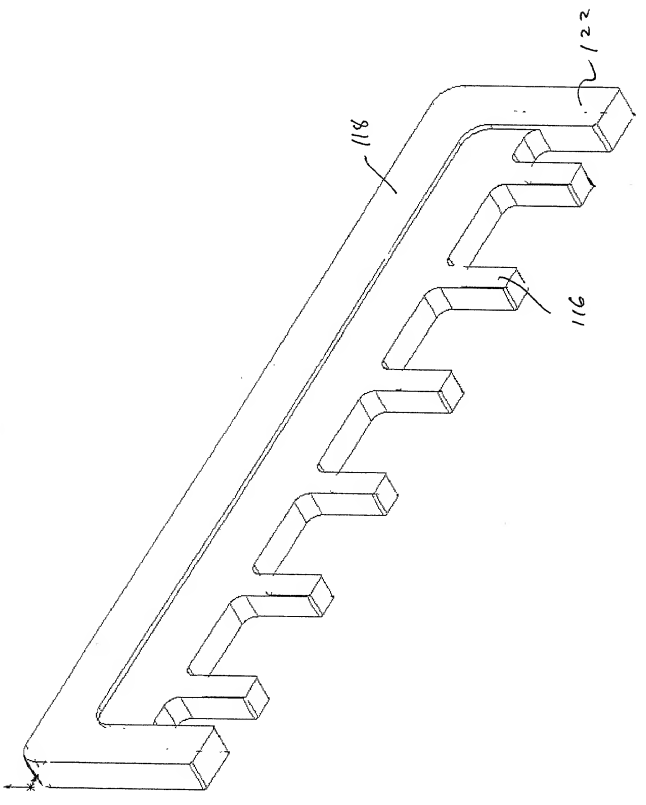


FIG. 20

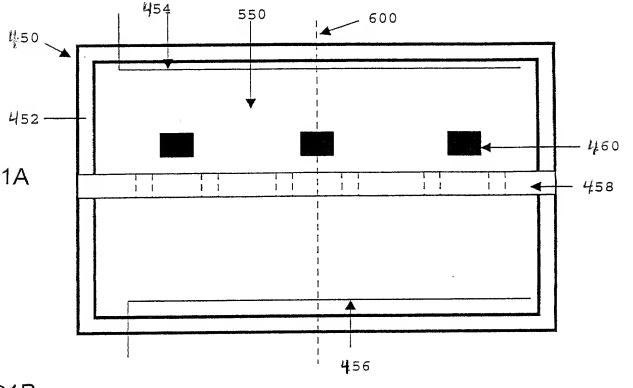


FIG. 21A

FIG. 21B

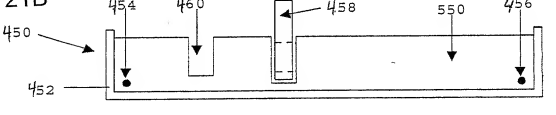
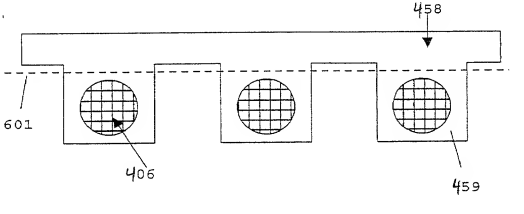
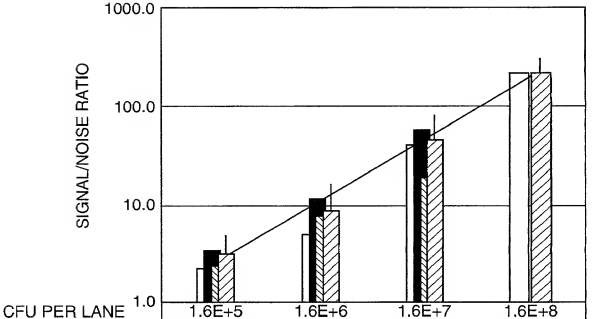


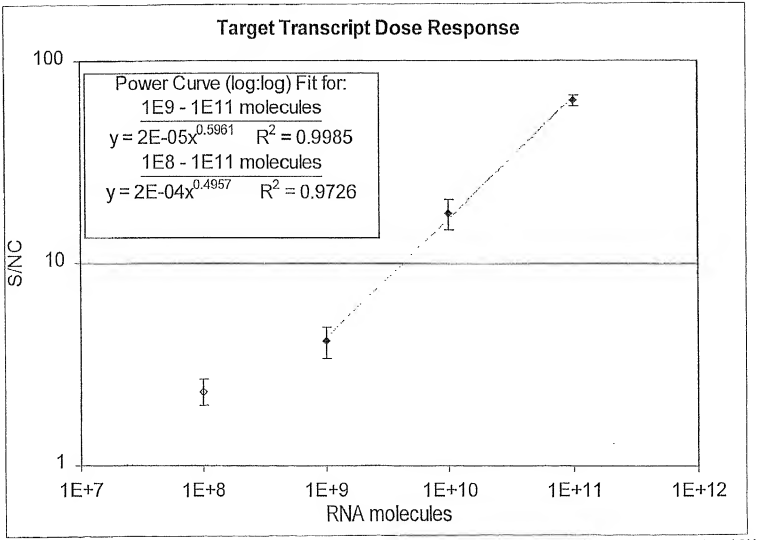
FIG. 21C





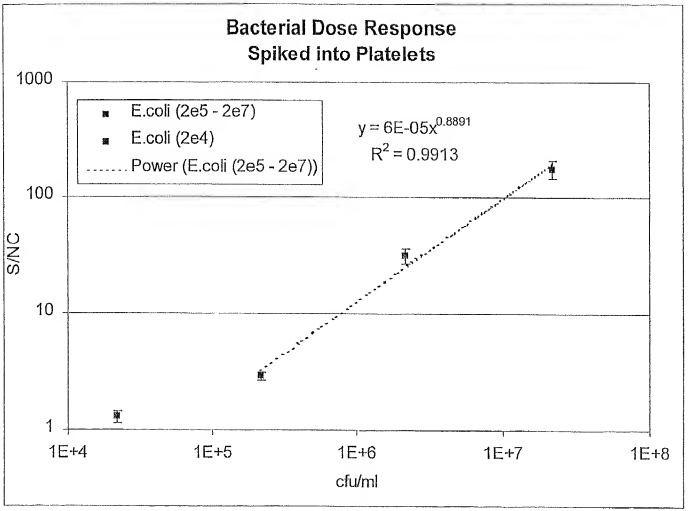
CFU PER LANE	1.6E+5	1.6E+6	1.6E+7	1.6E+8
ASSAY 1	2.2	4.9	41.3	214.7
ASSAY 2	3.5	11.6	58.3	
ASSAY 3	2.4	7.9	19.4	
OVERALL MEAN	3.1	8.8	46.0	214.7

FIG. 22



- S/N from target transcripts spiked into assay after centrifugation step (mean +/- s.e. (n=13))
- Copy# of 4.5S in *E.coli* ~1,000/cfu

FIG. 23



- S/N from *E.coli* (n=8) spiked into negative platelets, then processed according to the assay scheme (mean +/- s.e.).

FIG. 24

Assay Time

Sample Processing	
Target Enrichment	2min
Rinse	2min
Lysis	6min
Cool	2min

Assay	
Probe Hybridization	11min
Electrophoretic Capture	20min
Wash	5min
Detection	10min

12 - 15 minutes

Centrifuge samples x 1min
Uncap, pour off liquid - BIOHAZARD
Pipet Rinse Buffer
Vortex to resuspend
Centrifuge samples x 1min
Uncap, pour off liquid - BIOHAZARD
Pipet Lysis Buffer
Vortex to resuspend
Obtain NC and PC tubes
Place NC, PC and sample tubes in heater
Incubate through heat-cool cycle
5min at >100C => 2min to 45C

45 - 50 minutes

Pipet Hybridization Buffer
Mix by inversion several times
Incubate 10min at 45C

Pipet 50ul from each tube into cassette
Electrophorese for 20min

Move comb to Wash Buffer 1
Move comb to electroph wash slot
Electrophorese for 3min
Move comb to Conditioning Buffer
Dry comb
Pipet Substrate
Load Reader
Results Displayed

FIG. 25